Mutational and Physiologic Responses of *Habrobracon* in Biosatellite II[1]

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For our investigation of the genetic effects of spaceflight in Biosatellite II we elected to follow six principles: (1) It was essential to use mutational tests that had been used numerous times and ones in which we had considerable faith. The journey into outer space was innovative enough without adding innovations to the biologic test system itself. (2) Our test, in greatest depth, should be restricted to the most homogeneous population of cells, and in this case we chose the sperm for progeny testing. (3) We wished to look at the immediate genetic effects on the cells that had been exposed directly to the space environment and not at later generations of cells. (4) We wanted to test as many different cell types as possible because cells in different stages of gametogenesis not only have vastly different nucleocytoplasmic ratios (from sperm with a ratio $>$ 10:1 to mature oocytes with ratio of about 10^{-6}) but also are metabolically different and show different sensitivities to radiation. (5) We were determined to study dose-action kinetics of the organisms placed in a variety of positions away from the radiation source that was placed aboard Biosatellite II. Thus, we would have dose-action curves at our disposal, and we would have a series of doses of radiation for cells of varying sensitivities, with different dose optima for studying effects. Further, with a variety of containers in different positions in the spacecraft, we could look for position effects within the spacecraft itself. (6) Last, we resolved to keep a sharp lookout for anomalies that might arise which had no relation to the sensitive genetic tests we were to use — thus different physiologic criteria also were assayed.

The parasitic wasp, *Habrobracon,* is especially suitable for the types of genetic studies that were performed in Biosatellite II. This small braconid, *Habrobracon juglandis* Ashmead = *Bracon hebetor* (Say), has been the object of genetic investigations for 50 years and has been particularly useful for mutational analyses. It has a short life cycle (approximately 9 days at 30 C), a visible ontogeny (embryonic development is clearly seen through a transparent chorion; the larvae are ectoparasitic; and the pupae dwell in a brittle, easily breakable cocoon), and staging and sensitivity of the gametes have been thoroughly studied and are well understood. One of its more useful features is that unfertilized eggs develop into haploid males, a phenomenon that simplifies dominant lethal analyses and facilitates identification of mutation changes such as recessive lethals and translocations occuring any place in the genome.

A feature of *Habrobracon* that has been exploited during the last two decades is that information on the entire genome at every stage of development can be obtained from every egg laid by a treated female, or by a female mated with a treated male. In order that each egg be followed throughout development, there necessarily is a limit to the

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number of females (about 150 for each day of egg laying) that any one investigator can handle. Since the flight of the Biosatellite II was guaranteed to be a unique experience, we were pleased to have the help of a number of investigators from several different institutions (von Borstel et al., 1968). We worked together for a short time before, during, and after the flight and the postflight tests in order to secure large volumes of data.

EXPERIMENT DESIGN AND METHODS

Experiments

The data reported here were obtained from three experiments with *Habrobracon* in the spacecraft, one from the orbital flight and two from the tests on Earth after the flight. Each of these experiments in the spacecraft was controlled by the *Habrobracon* being exposed to radiation in a setup which was of essentially the same geometrical configuration as the flight vehicle. The postflight tests afforded the opportunity to examine the animals exposed inside the spacecraft when it was not subjected to stress (the phase B test), (cf. paper by Saunders, Reynolds, and Smith, p. 1) and after it had been subjected to vibration, acceleration, and shock that approximated the stresses of the flight itself (the phase C test). Since preparation for a *Habrobracon* experiment requires a 43-day leadtime to build up the genetic stocks necessary for a test and a 6-month posttest period for the numerous genetic analyses of the organisms, we were unable to analyze all of the organisms placed in the flight vehicle and Earth-based control setup during the test phases B and C. Therefore, it was necessary for us to make guesses as to which postflight tests were most crucial—we guessed correctly most of the time.

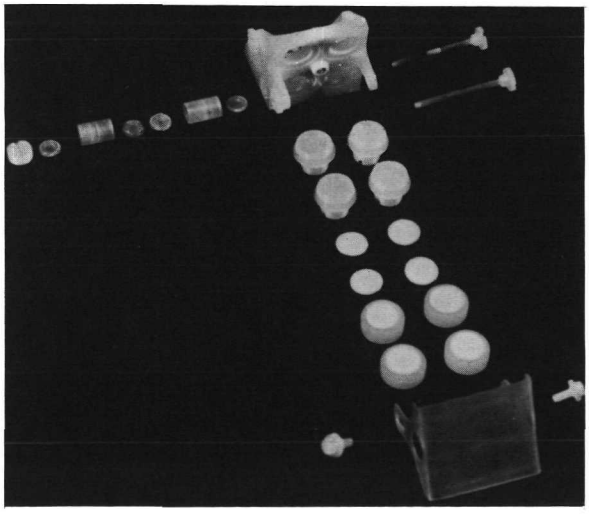

FIGURE 1.— Disassembled flight package used for the *Habrobracon* portion of the Biosatellite II experiment.

Hardware

Five packages, each containing four modules to hold the *Habrobracon,* were used for the experiment. The modules were secured on each package to face the radiation source and named according to their position facing the source; i.e., LR is lower right, UL is upper left, etc. A disassembled pack-

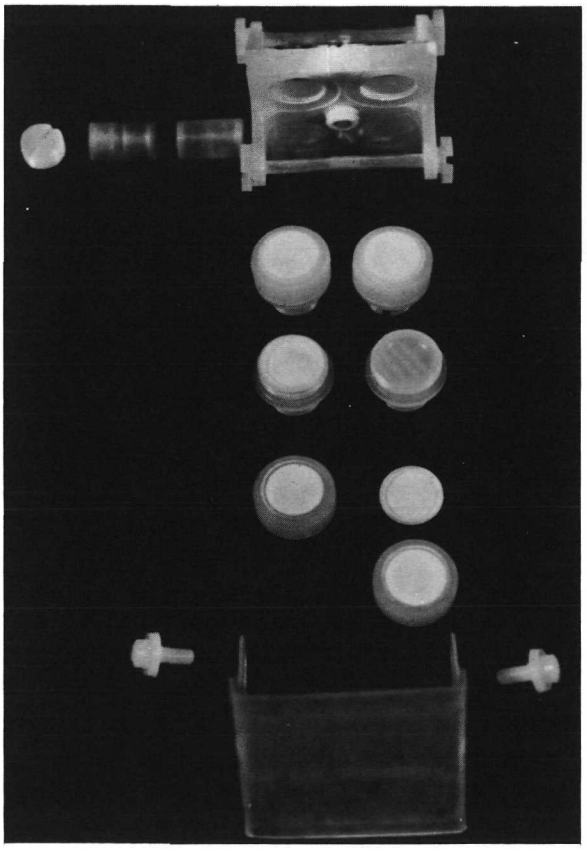

FIGURE 2.— Partially assembled flight package. The tubes for lithium fluoride powder are in place on the bracket; two of the modules are capped; one of the modules has a screen overlying the depression where the *Habrobracon* are held; and the other module is still disassembled. The capped containers for the *Artemia* and *Saccharomyces* are at the upper left of the photograph.

age used for the *Habrobracon* experiment is shown in figure 1. The same package, in different degrees of assembly, is shown in figures 2 and 3. Three of the packages were like the one shown in the figures. The 4000 R nominal exposure package had a concave face for placement of the modules containing the *Habrobracon* to ensure adherence to the isodose requirements. The 0 R nominal exposure package held multilayered modules so that more *Habrobracon* could be contained. Some of the *Habrobracon* in this package were irradiated before the launch.

So that the radiation exposure could be measured, each module carried three Toshiba glass-rod dosimeters. Additional dosimetry was accomplished by placing lithium fluoride (LiF) powder in tubes held in front of and behind the modules. *Artemia salina* (San Francisco strain) and *Saccharomyces cerevisiae* were placed in the containers provided for them at the lower part of the packages. A thermistor to record the local temperature was located centrally between the modules.

Radiation

Most of the *Habrobracon* were exposed to five (including 0 R) different exposures of radiation during the flight. Some of the *Habrobracon* were irradiated with 2000 R at a rate of exposure of approximately 120 R/min just before the launch; these were held in the nonradiation section of the flight vehicle. The *Saccharomyces* was placed in the package primarily to detect scattered radiation, since its response to linear energy transfer is well worked out (Mortimer, Brustad, and Cormack, 1965). *Artemia* blastocysts were used, since they are sensitive to vibration.

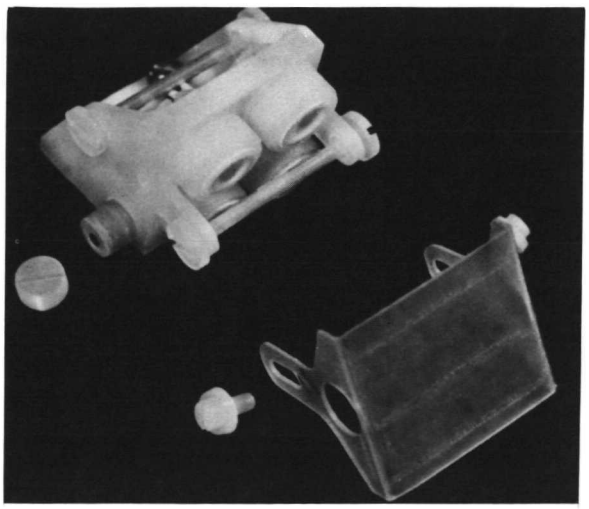

FIGURE 3.— Partially assembled flight package. The modules and tubes for lithium fluoride powder are in place; one of the containers for the *Artemia* and *Saccharomyces* is shown protruding from the side of the bracket. The secondary electron equilibrium shield lies in front of the package.

The five packages in the spacecraft and in the Earth-based control system were placed relative to the 85-strontium source so that the *Habrobracon* would receive nominal γ-radiation exposures of 4000, 2000, 1000, 500, and 0 R. The highest exposure was not achieved in Biosatellite II because of the foreshortened flight. By an unfortunate circumstance the 1000 R nominal exposure package was tilted in the spacecraft. The bracket holding it had been inadvertently turned 180° during spacecraft assembly so that the package was tilted upward at an angle of approximately 30° away from the source. This mistake was not noted and therefore not corrected by any of the inspectors who were responsible for checking the assembly of the spacecraft. It was detected after the flight when the Toshiba glass dosimeters and LiF powder were being measured. The error was verified by examination of photographs taken during disassembly of the spacecraft. This configuration of the 1000 R package was used only in the Biosatellite II flight and in the flight vehicle in the phase C experiment.

For the Biosatellite II flight, the radiation source was activated at 7:04 p.m. EDT on September 7 and closed on the 28th orbit at 12:58 p.m. EDT on September 9, an exposure period of nearly 42 hours. This exposure duration was also used in the phase B and phase C tests. The average exposures calculated for the *Habrobracon* positions inside the modules are shown in table 1.

Habrobracon

The strains of *Habrobracon* used for the experiments were selected for their divers properties (table 2). The Raleigh (*R*) strain, wild type, is vigorous and long lived under conditions of stress. The *lemon* strain has fragile females and reasonably vigorous males, but the *lemon* locus has the useful characteristics of being semidominant. By the time the Biosatellite II vehicle was ready to be launched, we had decided to incorporate a linkage

TABLE 1. – Radiation Conditions (Corrected for Geometry and Scattered Radiation) in the Flight Vehicle and Earth-Based Control

Experiment	Average exposure to *Habrobracon*, R				
Nominal exposure	0	500	1000	2000	4000
Biosatellite II flight	.7	356	589	1272	2425
Biosatellite II Earth control I	1.7	371	729	1245	2431
Phase B flight	1.5	342	689	1219	2376
Phase B Earth control I	1.5	341	723	1261	2442
Phase C flight	1.7	352	575	1232	2471
Phase C Earth control I	1.5	360	726	1241	2420

TABLE 2. – *Habrobracon* Strains Used in Flight and Earth-Based Experiments

[*R*, Raleigh wild-type strain; *le*, lemon strain; *c*, cantaloupe; *ho*, honey]

Date	Experiment	Animals in test		Females used for mating
		Females	Males	
Sept. 7 to 9, 1967	Biosatellite II	$\frac{le++}{+\,c\ ho}$	*le*	*R*+/+
Mar. 12 to 14, 1968	Phase B	$\frac{le++}{+\,c\ ho}$	*R*+	$\frac{c\ ho}{c\ ho}$
May 22 to 24, 1968	Phase C	$\frac{c\ ho}{c\ ho}$	*R*+	$\frac{c\ ho}{c\ ho}$

test into our program and had prepared vigorous females, heterozygous for the *lemon (le), cantaloupe (c)*, and *honey (ho)* markers on chromosome I.

Since the phase B and C tests were done shortly after the flight, we were unable to build up the *lemon* strain to a sufficiently large population without jeopardizing the data collecting from the flight animals. We elected to use males from the Raleigh strain for phases B and C and to use our supply of *lemon* males to prepare the heterozygous females. By the time of the phase C test, we were unable even to prepare the heterozygous females without jeopardizing the collection of data from the phase B test. Both post-flight tests followed the flight too closely for optimum control of all parameters of the *Habrobracon* experiment. Nevertheless, by making deliberate choices in one postflight test or the other, we were able to control fully all of the endpoints where spaceflight effects had been found.

Analysis of the males followed the procedures of von Borstel and Rekemeyer (1959) for dominant lethality, recessive lethality, and inherited partial sterility. Analysis of the females followed the procedures of Whiting, Smith, and von Borstel (1968) for total dominant and recessive lethality induced during oogenesis, and of Grosch (1968) for oogonial killing and dominant lethality.

Yeast

The strain X841 yeast is a diploid yeast of the following genetic composition:

$$\frac{a}{a}\ \frac{his5\text{-}2}{his5\text{-}2}\ \frac{trp1\text{-}1}{trp1\text{-}1}\ \frac{+}{pet1}\ \frac{arg4\text{-}1}{ARG4\text{-}1}\ \frac{ARG4\text{-}2}{arg4\text{-}2}\ \frac{thr1}{THR1}\ \frac{met1}{MET1}\ \frac{URA1}{ura1}\ \frac{lys1}{LYS1}\ \frac{LEU1}{leu1}\ \frac{ADE2}{ade2}$$

This multipurpose stock has a variety of uses (Mortimer et al., 1965). Since most of the experiments for which this stock was designed were already being carried out in the Biosatellite II experiment, we were interested primarily in two tests: (1) A determination of the frequency of recombination within the *arg4* gene, as estimated by the frequency

of revertant colonies on plates lacking arginine but containing all other auxotrophic requirements; and (2) a determination of the frequency of recombination between the *ade2* marker and the centromere, as estimated by the frequency of red colonies and colonies with red sectors.

The yeast was grown at 30 C in 600 ml of complete medium in four 2000-ml flasks which were constantly shaken. They were prepared about 2 to 3 weeks prior to flight. After the yeast growth had saturated at approximately 2×10^8 cells/ml, the flasks were sampled to determine which had the lowest frequency of revertants. These flasks were stored at 2 C.

Two days prior to the flight the yeast suspensions were centrifuged and the supernatant fluid was decanted. The wet yeast was then packed in the small cylindrical containers shown in figure 1 and again refrigerated. The yeast was transported to Cape Kennedy under refrigeration. After termination of the experiment, the yeast was brought back to the laboratory under refrigeration. Assays were carried out on complete medium and on medium containing all requirements except arginine.

Artemia

The San Francisco strain of *Artemia salina* was used for the Biosatellite II experiment. It has the

TABLE 3. — Summary of Dominant Lethality in *Habrobracon* Sperm in the Biosatellite II Experiment

Observation	Nonirradiated packages		Radiation packages			
Nominal exposures, R	0	2000 X-ray	500	1000	2000	4000
Biosatellite II flight						
Actual exposures, R	0.7	2000	356	589	1272	2425
Total eggs	3337	704	2010	1691	1536	1736
Preblastula death	46	177	107	160	308	538
Male offspring	925	236	574	432	423	564
Female offspring	1628	200	869	781	570	411
Frequency of preblastula death of diploid eggs	0.015	0.421	0.076	0.133	0.298	0.509
Biosatellite II Earth control						
Actual exposures, R	1.7	2000	371	729	1245	2431
Total eggs	2309	865	1705	1782	1512	1712
Preblastula death	30	252	119	176	249	552
Male offspring	702	270	524	601	536	587
Female offspring	1186	234	765	732	495	359
Frequency of preblastula death of diploid eggs	0.014	0.466	0.105	0.161	0.284	0.550
Biosatellite II postflight phase B flight vehicle						
Actual exposures, R	1.5	2000	34.2	689	1219	2376
Total eggs	722	739	704	618	582	730
Preblastula death	13	248	38	60	106	276
Male offspring	176	166	146	153	132	184
Female offspring	454	241	440	310	257	201
Frequency of preblastula death of diploid eggs	0.014	0.444	0.062	0.125	0.237	0.522

highest frequency of emergence for any wild-type strain, with approximately 77 percent of the cysts developing to the adult stage. The *Artemia* cysts were loaded from the vacuum-sealed containers in which they are kept into the cylindrical containers used in the experiment (fig. 1). Some of the *Artemia* were preirradiated with 16 kR. Six replicate dishes with 50 cysts per dish were assayed at each dose level.

RESULTS

The first observation made after the flight of Biosatellite II was that every *Habrobracon* had survived and all were in excellent condition.

Genetic Analysis of Males

There were no differences among the flight- and Earth-based controls when spontaneous or radiation-induced dominant lethal mutations in sperm were the criteria (table 3). Table 3 contains the computed frequencies of the preblastula deaths, the class that represents deaths caused by the breakage-fusion-bridge cycle (von Borstel, 1960). Other types of dominant lethal events such as those caused by large deletions or losses of chromosomes were present in the same frequency in the flight and control animals. This is shown in figure 4 where dose-action curves for hatchability are presented; hatchability represents that fraction of the population which escapes death both from bridge breakage and from chromosome imbalance.

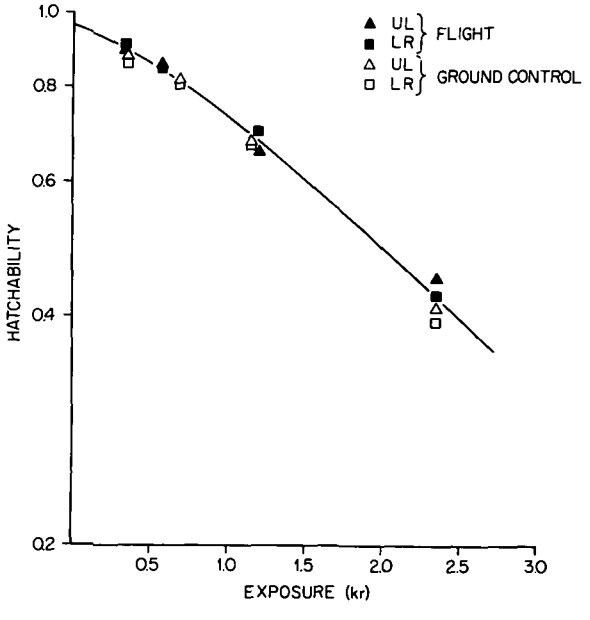

FIGURE 4.— Hatchability of fertilized eggs from females mated to males irradiated in the spacecraft and the Earth-based control. UL, *Habrobracon* from upper left modules; LR, *Habrobracon* from lower right modules (von Borstel et al., 1968).

The spontaneous recessive lethal mutation frequency in sperm in the flight animals was 22/378 = 0.058 (table 4). This was greater by a factor of more than 3 than that of the controls, 12/732 = 0.016.[2] Most of the control data were obtained from the same strains of *Habrobracon* tested before the launch. In the phase C experiment spontaneous lethal mutation frequency in sperm showed an enhancement similar to that found in flight animals when compared with that of animals in the phase B experiment. The phase C animals were from the flight vehicle which had undergone a simulation of the flight profile. Thus, we believe that the enhancement of spontaneous recessive lethal frequency in sperm was induced by some factor of the flight profile other than weightlessness.

There was no effect of spaceflight on radiation-induced recessive lethal mutation frequency or on frequency of inherited partial sterility in sperm (table 4 and fig. 5). The recessive lethal mutation frequencies follow the expected one-hit kinetics, and inherited partial sterility approaches the expected two-hit kinetics.

Genetic Analysis of Females

The females were transferred at daily or subdaily intervals for a 20-day period. The transfers were made at previously established intervals and were designed to permit collection of groups of eggs which were in different stages of oogenesis during the flight.

One of the more puzzling findings in the Biosatellite II experiment was the lethality induced in the *Habrobracon* oocytes which were in the first meiotic metaphase during the spaceflight (table 5). Though fecundity was higher for the metaphase I oocytes from nonirradiated females in the flight vehicle than for those from nonirradiated females in the Earth-based control (379 and 298 eggs, respectively), the viability was distinctly lower (0.491 compared with 0.893). A similar circumstance was obtained for metaphase I oocytes from

[2] Pooled control data from these and other sources.

TABLE 4. – Summary of Recessive Mutations and Translocations in Sperm in Biosatellite II Tests

Observation	Nonirradiated packages				Preirradiated packages		Irradiated packages with nominal dose, R, of —											
					2000		500		1000			2000			4000			
	Flight	Earth control I	Phase B	Phase C	Flight	Earth control I	Flight	Earth control I	Flight	Earth control I	Phase B chronic	Flight	Earth control I	Phase B chronic	Flight	Earth control I	Phase B chronic
Actual dose, R	0.7	1.7	1.5	1.7	2000	.....	356	371	589	729	689	1272	1245	1219	2425	2431	2376
Recessive lethals:																	
Embryonic	1/378	0/73	0/172	2/354	7/86	5/90	4/362	5/137	5/336	4/76	5/202	15/292	5/64	9/179	22/291	20/182	10/146
	(0.003)	(0.0)	(0.0)	(0.006)	(0.081)	(0.056)	(0.011)	(0.036)	(0.015)	(0.053)	(0.025)	(0.051)	(0.078)	(0.050)	(0.076)	(0.110)	(0.068)
Larval	10/378	2/73	3/172	10/354	10/86	12/90	9/362	8/137	15/336	8/76	12/202	25/292	5/64	20/179	51/291	23/182	25/146
	(0.026)	(0.027)	(0.017)	(0.028)	(0.116)	(0.133)	(0.025)	(0.058)	(0.045)	(0.105)	(0.059)	(0.086)	(0.078)	(0.112)	(0.176)	(0.126)	(0.171)
Pupal	11/378	0/73	0/172	5/354	13/86	12/90	15/362	6/137	8/336	5/76	1/202	21/292	3/64	11/179	39/291	17/182	5/146
	(0.029)	(0.0)	(0.0)	(0.014)	(0.151)	(0.133)	(0.041)	(0.044)	(0.024)	(0.066)	(0.005)	(0.072)	(0.047)	(0.061)	(0.134)	(0.093)	(0.034)
Visible mutations	0/378	0/73	0/172	0/354	0/86	0/90	0/362	0/137	1/336	0/76	1/202	2/292	0/64	1/179	8/291	5/182	2/146
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.003)	(0.0)	(0.005)	(0.007)	(0.0)	(0.006)	(0.027)	(0.027)	(0.014)
Translocations:																	
Total	0/378	0/73	1/172	1/354	14/86	10/90	5/362	3/137	5/336	4/76	7/202	12/292	3/64	16/179	52/291	31/182	35/146
	(0.0)	(0.0)	(0.006)	(0.003)	(0.163)	(0.111)	(0.014)	(0.022)	(0.015)	(0.053)	(0.035)	(0.041)	(0.047)	(0.089)	(0.179)	(0.170)	(0.240)
Females with one or more	0/378	0/73	1/172	1/354	11/86	10/90	5/362	2/137	5/336	4/76	5/202	11/292	3/64	15/179	48/291	28/182	30/146
	(0.0)	(0.0)	(0.006)	(0.003)	(0.128)	(0.111)	(0.014)	(0.015)	(0.015)	(0.053)	(0.025)	(0.038)	(0.047)	(0.084)	(0.165)	(0.154)	(0.205)
With position effects	0/378	0/73	1/172	0/354	7/86	4/90	1/362	0/137	2/336	1/76	5/202	4/292	1/64	9/179	35/291	22/182	20/146
	(0.0)	(0.0)	(0.006)	(0.0)	(0.081)	(0.044)	(0.003)	(0.0)	(0.006)	(0.013)	(0.025)	(0.014)	(0.016)	(0.050)	(0.120)	(0.121)	(0.137)
Total wasps tested	378	73	172	354	86	90	362	137	336	76	202	292	64	179	291	182	146

TABLE 5. – Summary of Survival of Offspring from Oocytes Irradiated in the First Meiotic Metaphase Stage (Transfer 1)

Observation	Nonirradiated package with module-layer preirradiation exposure, R, of—						
	Outer, 0	Middle, 0	Inner, 0	Outer, 0	Middle, 0	Inner, 2000	Pooled, 0
	UR			LL			
Flight							
Actual exposure, R						2000	0.7
Total eggs	85	81	68	69	76	147	379
Eggs hatched	81	55	50	67	74	114	327
Hatchability	0.953	0.679	0.735	0.971	0.974	0.776	0.863
Stages of death:							
1	0	1	0	0	1	15	2
3	3	6	9	2	0	9	20
4	0	7	4	0	0	4	11
5	1	12	5	0	1	5	19
Adults eclosed	40	21	9	57	59	83	186
Adult survival	0.494	0.259	0.132	0.826	0.776	0.565	0.491
Earth control I							
Actual exposure, R						2000	1.7
Total eggs	99	53	18	62	66	95	298
Eggs hatched	96	49	17	57	61	91	280
Hatchability	0.970	0.925	0.944	0.919	0.924	0.958	0.940
Stages of death:							
1	0	0	0	0	2	3	2
3	3	2	1	3	1	0	10
4	0	1	0	2	1	0	4
5	0	1	0	0	1	1	2
Adults eclosed	94	48	15	54	55	88	266
Adult survival	0.949	0.906	0.833	0.871	0.833	0.926	0.893

Observation	Radiation packages with nominal exposure, R, of —											
	500			1000			2000			4000		
	UR	LL	Pooled	UR	LL	Pooled	UR	LL	Pooled	UR	LL	Pooled
Flight												
Actual exposure, R			356			589			1272			2425
Total eggs	35	48	83	103	104	207	77	94	171	87	99	186
Eggs hatched	31	44	75	44	65	109	24	8	32	11	10	21
Hatchability	0.886	0.917	0.904	0.427	0.625	0.527	0.312	0.085	0.187	0.126	0.101	0.113
Stages of death:												
1	2	1	3	3	1	4	9	0	9	18	0	18
3	0	1	1	13	13	26	32	28	60	54	38	92
4	1	2	3	13	5	18	2	40	42	0	51	51
5	1	0	1	30	20	50	10	18	28	4	0	4
Adults eclosed	8	17	25	9	12	21	17	0	17	8	5	13
Adult survival	0.229	0.354	0.301	0.087	0.115	0.101	0.221	0.0	0.099	0.092	0.051	0.070
Earth control I												
Actual exposure, R			371			729			1245			2431
Total eggs	51	53	104	82	57	139	72	31	103	43	61	104
Eggs hatched	46	47	93	64	44	108	42	18	60	12	16	28
Hatchability	0.902	0.887	0.894	0.780	0.772	0.777	0.583	0.581	0.583	0.279	0.262	0.269
Stages of death:												
1	1	3	4	12	5	17	17	8	25	19	23	42
3	1	0	1	3	8	11	10	3	13	8	20	28
4	2	0	2	3	0	3	2	1	3	2	2	4
5	1	3	4	0	0	0	1	1	2	2	0	2
Adults eclosed	38	38	76	59	36	95	36	17	53	10	13	23
Adult survival	0.745	0.717	0.731	0.720	0.632	0.683	0.500	0.548	0.515	0.233	0.233	0.213

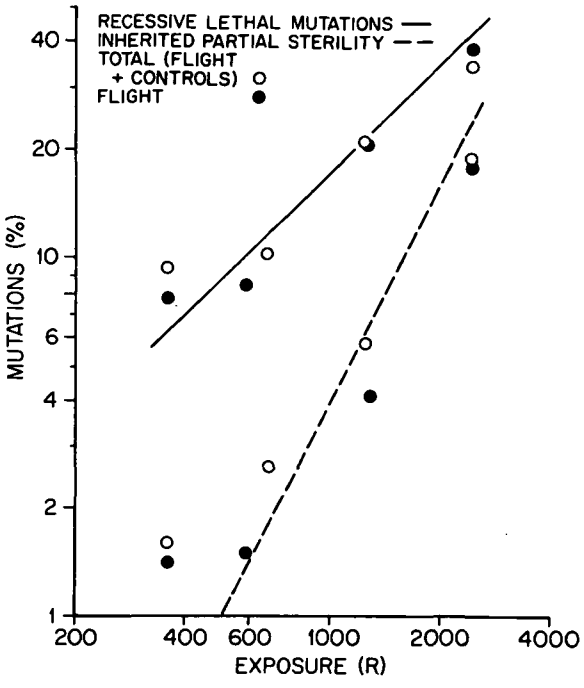

FIGURE 5.— Dose-action curves for total recessive lethal mutations and inherited partial sterility in sperm.

females exposed to 2000 R of X-radiation before the flight; fecundity was 147 and 95 and viability was 0.565 and 0.93, respectively.

The stages at which the developing *Habrobracon* died are shown in figure 6. It can be seen that, with the exception of the earliest stage, the distribution of deaths throughout development is relatively uniform for the metaphase I oocytes from the nonirradiated flight animals. Death at stage 1 connotes dominant lethality, usually from induction of the breakage-fusion-bridge cycle. This was noted in the data for the metaphase I oocytes from the flight animals irradiated before the flight.

An increase in frequency of deaths at stage 3 usually indicates death of the embryo from chromosome imbalance. This can be seen from the distribution of stages of death for unweighted averages of stages of death of offspring from females heterozygous for translocations (fig. 6). The unweighted averages of stages of death of offspring from 50 females heterozygous for recessive lethal mutations shown on the same diagram indicate that a reasonably uniform distribution for times of death is obtained from stage 4 throughout the rest of development.

Thus, a case can be made that the excess of deaths found among the offspring from females that had been in the flight vehicle may be from a mixture of chromosome imbalance phenomena and recessive lethal mutations induced by the spaceflight conditions. This reduction in survival was dependent upon the location of the nonirradiated females both in the package and in the module; those in the innermost and middle layers in the upper right module had the lowest hatchabilities. A similar pattern is shown in table 6 for the slightly younger oocytes that had not been subject to irradiation during the flight. Thus, even though these results were not found in the phase C test, we believe that the responses in the unirradiated *Habrobracon* females, which differed from one position to another within the space vehicle, indicate that the low viability and apparent increase in mutation frequency came from some spaceflight stress other than weightlessness. It can be seen that the metaphase I oocytes of the flight females irradiated either before or during the flight generally had significantly lower hatchabili-

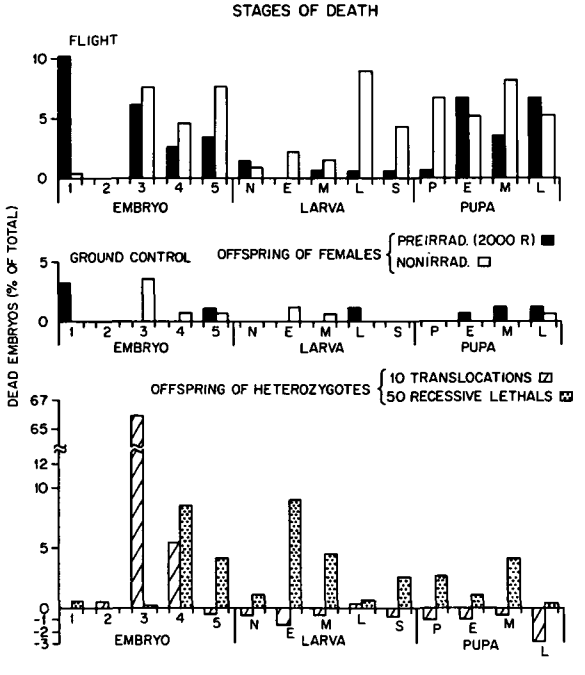

FIGURE 6.— Stages of death of *Habrobracon* from eggs which were in metaphase I in the spacecraft and in the Earth-based control, and stages of death of eggs from a random sample of females heterozygous for recessive lethal mutations and translocations.

ties than those laid by the females from the Earth-based control setup (table 5 and figs. 6 and 7). This is not true for the slightly younger stages (table 6) in which, if anything, the flight oocytes were slightly more resistant to radiation than those in the Earth-based control.

The oocytes in the diplotene stage of the meiotic prophase were quite resistant to any effects of the flight or the concomitant irradiation (tables 7 and 8); thus effects of spaceflight factors on the ooplasm and yolk are excluded.

Data from the phase B and C tests are not listed, since they followed closely those for the females in the Earth-based control.

Hatchability data similar to those compiled in tables 5 to 8 were obtained for all 20 days of the egg-laying period. The data for one exposure only (the highest) are summarized in figures 8 to 11. The day-by-day fecundity data for the Earth-control animals are presented in figure 8. These data clearly show that the fecundity-depressing effects of radiation are obliterated and even reversed in those animals exposed to spaceflight conditions. That is, more eggs than normal were obtained from the oogonia irradiated during the flight. Even the animals which were irradiated before the flight had higher fecundity than the preirradiated females that were kept on Earth; the preirradiated females did not exhibit the higher-than-normal egg production shown by those irradiated during the flight. The animals exposed to the

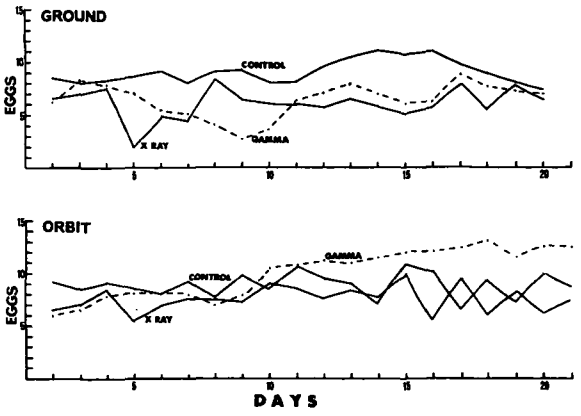

FIGURE 8.— Mean daily egg production for samples of *Habrobracon* virgin females from the spacecraft and the Earth-based control. In each case, the curves have been drawn for unirradiated controls, for females receiving an acute preflight exposure of 2000 R of X-radiation, and for females receiving 2425 R of γ-radiation during the flight itself (from Grosch, 1968).

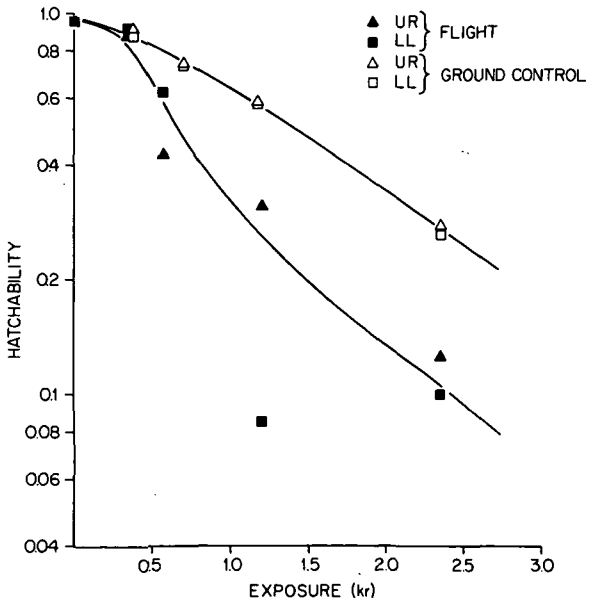

FIGURE 7.— Hatchability of unfertilized eggs from virgin females irradiated in the spacecraft and the Earth-based control. The eggs represent the class that was in the first meiotic metaphase at the time of irradiation. UR, *Habrobracon* from upper right modules; LL, *Habrobracon* from lower left modules (von Borstel et al., 1968).

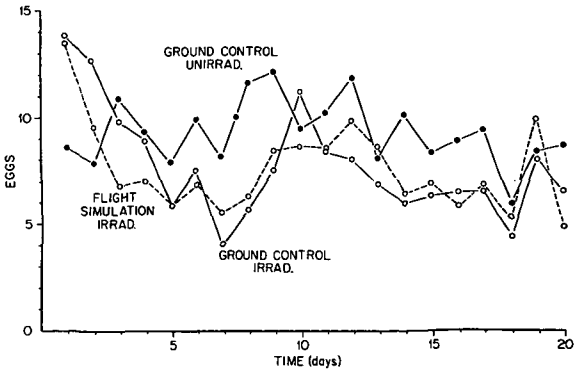

FIGURE 9.— Mean daily egg production for samples of *Habrobracon* virgin females from the spacecraft and the Earth-based control in the phase C test. The curves have been drawn for unirradiated control females, for females in the flight vehicle receiving 2471 R, and for females in the Earth-based control receiving 2420 R (from Grosch, 1970).

TABLE 6. – Summary of Survival of Offspring From a Mixture of Oocytes Irradiated in the First Meiotic Metaphase, Diakinesis, and Late Diplotene Stages (Transfers 2, 3, and 4)

Observation	Nonirradiated package with module-layer preirradiation exposure, R, of—						
	Outer, 0	Middle, 0	Inner, 0	Outer, 0	Middle, 0	Inner, 2000	Pooled, 0
	UR			LL			
Flight							
Actual exposure, R						2000	0.7
Total eggs	128	79	82	70	87	85	446
Eggs hatched	125	43	72	65	78	59	383
Hatchability	0.977	0.544	0.878	0.929	0.897	0.694	0.859
Stages of death:							
1	0	0	0	0	1	0	1
3	1	3	1	4	1	5	10
4	1	7	4	0	2	13	14
5	1	26	5	1	5	8	38
Adults eclosed	86	35	52	36	64	39	273
Adult survival	0.688	0.443	0.634	0.514	0.736	0.459	0.612
Earth control I							
Actual exposure, R						2000	1.7
Total eggs	63	61	62	77	71	122	334
Eggs hatched	62	56	61	76	69	117	324
Hatchability	0.984	0.918	0.984	0.987	0.972	0.959	0.970
Stages of death:							
1	0	2	0	1	1	2	4
3	1	2	0	0	0	1	3
4	0	0	1	0	1	1	2
5	0	1	0	0	0	1	1
Adults eclosed	51	47	44	52	44	66	238
Adult survival	0.810	0.770	0.710	0.675	0.620	0.541	0.713

Observation	Radiation packages with nominal exposure, R, of —											
	500			1000			2000			4000		
	UR	LL	Pooled	UR	LL	Pooled	UR	LL	Pooled	UR	LL	Pooled
Flight												
Actual exposure, R			356			589			1272			2425
Total eggs	73	37	110	77	106	183	110	112	222	145	113	258
Eggs hatched	52	28	80	74	100	174	97	89	186	98	74	172
Hatchability	0.712	0.757	0.727	0.961	0.943	0.951	0.882	0.795	0.838	0.676	0.655	0.667
Stages of death:												
1	1	0	1	1	0	1	0	10	10	12	18	30
3	1	0	1	2	3	5	9	4	13	34	17	51
4	4	1	5	0	2	2	2	1	3	0	1	1
5	15	8	23	0	1	1	2	8	10	1	3	4
Adults eclosed	34	16	50	59	81	140	64	68	132	67	48	115
Adult survival	0.466	0.432	0.455	0.766	0.764	0.765	0.582	0.607	0.595	0.462	0.425	0.446
Earth control I												
Actual exposure, R			371			729			1245			2431
Total eggs	52	58	110	54	66	120	103	103	206	118	131	249
Eggs hatched	50	57	107	47	54	101	75	77	152	56	73	129
Hatchability	0.962	0.983	0.973	0.870	0.818	0.842	0.728	0.748	0.738	0.475	0.557	0.518
Stages of death:												
1	0	0	0	3	3	6	15	12	27	39	26	65
3	2	0	2	1	7	8	11	10	21	17	24	41
4	0	1	1	0	2	2	1	2	3	5	8	13
5	0	0	0	3	0	3	1	2	3	1	0	1
Adults eclosed	41	48	89	32	33	65	54	48	102	44	51	95
Adult survival	0.788	0.828	0.809	0.593	0.500	0.542	0.524	0.466	0.495	0.373	0.389	0.382

TABLE 7. – Summary of Survival of Offspring From Oocytes Irradiated in Late Diplotene Stage (Transfer 5)

Observation	Nonirradiated package with module-layer preirradiation exposure, R, of—						
	Outer, 0	Middle, 0	Inner, 0	Outer, 0	Middle, 0	Inner, 2000	Pooled, 0
	UR			LL			
Flight							
Actual exposure, R						2000	0.7
Total eggs	96	129	57	88	88	78	458
Eggs hatched	94	125	57	88	82	76	446
Hatchability	0.979	0.969	1.000	1.000	0.932	0.974	0.974
Stages of death:							
1	1	3	0	0	0	0	4
3	0	0	0	0	0	2	0
4	0	0	0	0	0	0	0
5	1	1	0	0	6	0	8
Adults eclosed	86	114	51	80	81	73	412
Adult survival	0.896	0.884	0.895	0.909	0.920	0.936	0.900
Earth control I							
Actual exposure, R						2000	1.7
Total eggs	139	82	122	168	130	122	641
Eggs hatched	137	81	122	164	123	113	627
Hatchability	0.986	0.988	1.000	0.976	0.946	0.934	0.978
Stages of death:							
1	0	0	0	0	1	4	1
3	0	0	0	0	2	3	2
4	1	1	0	3	2	0	7
5	1	0	0	1	2	1	4
Adults eclosed	131	78	120	154	115	101	598
Adult survival	0.942	0.951	0.984	0.917	0.885	0.828	0.933

Observation	Radiation packages with nominal exposure, R, of —											
	500			1000			2000			4000		
	UR	LL	Pooled	UR	LL	Pooled	UR	LL	Pooled	UR	LL	Pooled
Flight												
Actual exposure, R			356			589			1272			2425
Total eggs	49	68	117	100	88	188	90	55	145	85	90	175
Eggs hatched	49	65	114	94	88	182	89	53	142	84	90	174
Hatchability	1.000	0.956	0.974	0.940	1.000	0.968	0.989	0.964	0.979	0.988	1.000	0.994
Stages of death:												
1	0	2	2	0	0	0	0	2	2	0	0	0
3	0	0	0	4	0	4	1	0	1	1	0	1
4	0	1	1	0	0	0	0	0	0	0	0	0
5	0	0	0	2	0	2	0	0	0	0	0	0
Adults eclosed	44	52	96	87	83	170	86	49	135	72	79	151
Adult survival	0.898	0.765	0.821	0.870	0.943	0.904	0.956	0.891	0.931	0.847	0.878	0.863
Earth control I												
Actual exposure, R			371			729			1245			2561
Total eggs	123	132	255	112	121	233	134	93	227	78	118	196
Eggs hatched	122	126	248	109	116	225	129	91	220	76	111	187
Hatchability	0.992	0.955	0.973	0.973	0.959	0.966	0.963	0.978	0.969	0.974	0.941	0.954
Stages of death:												
1	0	1	1	1	2	3	3	0	3	1	1	2
3	0	1	1	0	1	1	1	0	1	1	5	6
4	0	0	0	1	1	2	1	1	2	0	1	1
5	1	4	5	1	1	2	0	1	1	0	0	0
Adults eclosed	115	118	233	96	109	205	120	86	206	72	104	176
Adult survival	0.935	0.894	0.914	0.857	0.901	0.880	0.896	0.925	0.907	0.923	0.881	0.898

TABLE 8. – Summary of Survival of Offspring from Oocytes Irradiated in Diplotene Stage (Transfer 6)

Observation	Nonirradiated package with module-layer preirradiation exposure, R, of—						
	Outer, 0	Middle, 0	Inner, 0	Outer, 0	Middle, 0	Inner, 2000	Pooled, 0
	UR			LL			
Flight							
Actual exposure, R						2000	0.7
Total eggs	Not scored	57	86	Not scored	86	39	229
Eggs hatched	do	57	86	do	80	32	223
Hatchability	do	1.000	1.000	do	0.930	0.821	0.974
Stages of death:							
1	do	0	0	do	2	1	2
3	do	0	0	do	1	2	1
4	do	0	0	do	0	3	0
5	do	0	0	do	3	1	3
Adults eclosed	do	43	76	do	68	27	187
Adult survival	do	0.754	0.884	do	0.791	0.692	0.817
Earth control I							
Actual exposure, R						2000	1.7
Total eggs	130	162	91	58	69	86	509
Eggs hatched	118	160	91	56	68	70	493
Hatchability	0.908	0.988	1.000	0.966	0.986	0.814	0.969
Stages of death:							
1	0	0	0	0	0	8	0
3	0	0	0	1	1	6	2
4	0	2	0	1	0	1	3
5	12	0	0	0	0	1	12
Adults eclosed	110	152	81	36	52	60	431
Adult survival	0.846	0.938	0.890	0.621	0.754	0.698	0.847

Observation	Radiation packages with nominal exposure, R, of —											
	500			1000			2000			4000		
	UR	LL	Pooled	UR	LL	Pooled	UR	LL	Pooled	UR	LL	Pooled
Flight												
Actual exposure, R			356			589			1272			2425
Total eggs	Not scored	Not scored	Not scored	Not scored	Not scored	Not scored	71	71	142	64	63	127
Eggs hatched	do	do	do	do	do	do	71	65	136	58	62	120
Hatchability	do	do	do	do	do	do	1.000	0.915	0.958	0.906	0.984	0.945
Stages of death:												
1	do	do	do	do	do	do	0	0	0	3	0	3
3	do	do	do	do	do	do	0	4	4	3	1	4
4	do	do	do	do	do	do	0	0	0	0	0	0
5	do	do	do	do	do	do	0	2	2	0	0	0
Adults eclosed	do	do	do	do	do	do	65	58	123	42	51	93
Adult survival	do	do	do	do	do	do	0.915	0.817	0.866	0.656	0.810	0.732
Earth control I												
Actual exposure, R			371			729			1245			2561
Total eggs	76	131	207	44	31	75	40	85	125	67	77	144
Eggs hatched	76	128	204	44	30	74	39	78	117	58	69	127
Hatchability	1.000	0.977	0.986	1.000	0.968	0.987	0.975	0.918	0.936	0.866	0.896	0.882
Stages of death:												
1	0	0	0	0	1	1	1	0	1	5	3	8
3	0	1	1	0	0	0	0	4	4	2	4	6
4	0	1	1	0	0	0	0	2	2	1	0	1
5	0	1	1	0	0	0	0	1	1	1	1	2
Adults eclosed	64	110	174	32	26	58	31	67	98	45	54	99
Adult survival	0.842	0.840	0.841	0.727	0.839	0.773	0.775	0.788	0.784	0.672	0.701	0.688

flight simulation profile in the phase C experiment resembled the Earth-based control animals from the flight experiment (fig. 9).

Low hatchability frequencies seen in eggs which were in the early meiotic prophase during the flight (fig. 10) were not found in the Earth-based controls nor in the flight simulation experiment of phase C (fig. 11). It is of interest to note that the high survival of the irradiated oogonial cells in the flight, as seen by fecundity measurements (fig. 8), are also reflected in the hatchability measurements (fig. 10).

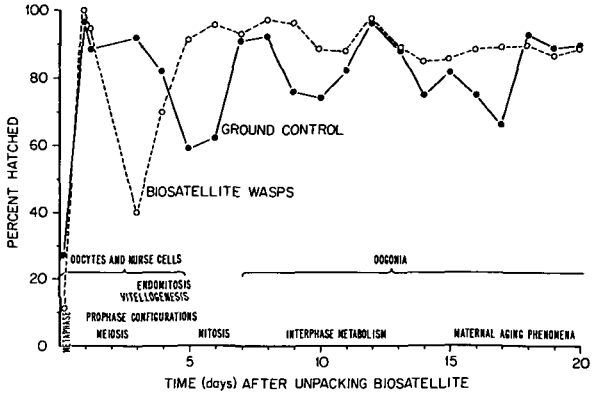

FIGURE 10.— Hatchabilities of eggs from *Habrobracon* virgin females from the Biosatellite II spacecraft (2425 R) and Earth-based control (2431 R). The hatchabilities are from each daily or subdaily transfer (from Grosch, 1970).

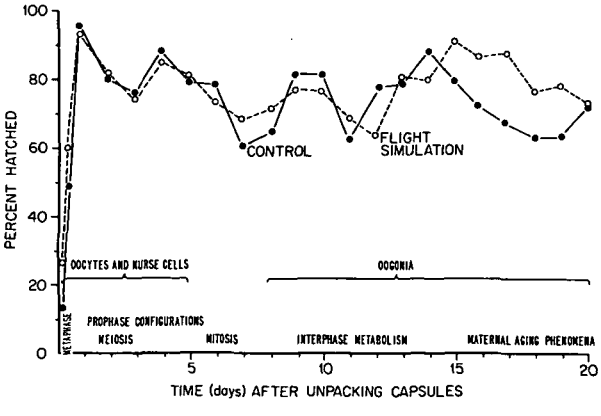

FIGURE 11.— Hatchabilities of eggs from *Habrobracon* virgin females exposed during the phase C test flight simulation (2471 R) and its control (2420 R) (from Grosch, 1970).

Genetic Recombination

Extensive data were accumulated on genetic recombination among markers on chromosome I of *Habrobracon*. These data are not included here because no effects of the spaceflight were found.

Observations on Behavior

The mating behavior of the males that had been in the spacecraft was altered. Observed matings of 243 males from the Earth-based control at Cape Kennedy were achieved in about 3 hours by three investigators. At Hickam Field it took three investigators over 13 hours to achieve with certainty one-third of the 254 planned matings of the flight animals. These males from the spacecraft were disoriented and unable to find or to copulate with the females. Most of the males must have recovered during the trip from Hickam Field to Oak Ridge because genetic studies indicated that 80.3 percent of the females had mated with the males from the spacecraft prior to being set for oviposition. Ninety-three percent of the males from the

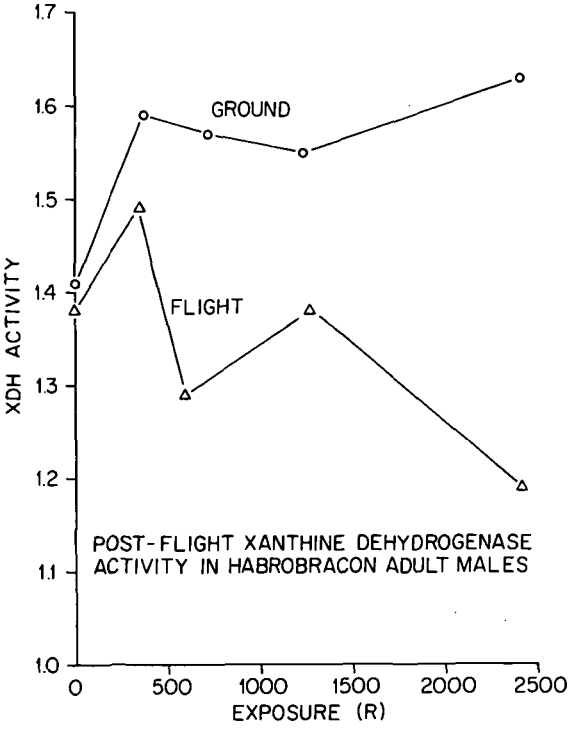

FIGURE 12.— Xanthine dehydrogenase (XDH) activity in the males from the spacecraft and Earth-based controls (Keller, unpublished data).

Earth-based control mated. This abnormal behavior was not observed in the postflight Earth-based control tests.

On the other hand, the *Habrobracon* females did not seem to be disoriented by their flight experience. When placed with host larvae, the females immediately began to paralyze them, feed, and lay eggs.

Physiologic and Late Effects

The fertilizing capacity of the sperm from the males in the spacecraft was greater than that of the sperm from males in the Earth-based control. The increased fertilizing capacity appears to be an enhancing effect of radiation combined with weightlessness, because the males not exposed to radiation and those exposed to the radiation prior to launch did not show this effect.

The *Habrobracon* males, after they had been mated to females, were frozen and shipped to Dr. E. C. Keller, who studied their xanthine dehydrogenase activity. He found that the activity of this enzyme in the flight animals was depressed more than that in the controls at all radiation levels (fig. 12 and unpublished data from Dr. Keller).

One of the most puzzling features of the Biosatellite II experiment was the data obtained for the longevity of females. The *Habrobracon* females that had been subjected to spaceflight conditions had a longevity approximately 10 percent greater than that of the controls (table 9). Although the females lived longer at every exposure to radiation, the data were not significant for any one exposure; taken altogether, however, the data showed a significant difference.

Saccharomyces

The assays used on the *Saccharomyces* were for intragenic and intergenic recombination. The diploid cells in the flight showed no difference for these two endpoints from those in the Earth control.

The survival curve for diploid yeast has a long shoulder, so that cell killing from radiation does not begin until an exposure of 15 kR is reached. The highest exposure for the yeast in the Biosatellite II experiment was 1516 R.

The data for induced intragenic recombination within the *arg4* locus are presented in table 10. It can be seen that there is essentially no difference in frequency at any exposure for yeast in the ground-based control and in the spacecraft.

The data for induced intergenic recombination are shown in table 11. These values show that at only one exposure (other than 0 R) did fewer red variants appear in the yeast that were in the Earth-based control than in those in the flight. The 95-percent confidence limits for the control and flight data do not overlap at the 900 R exposure. All the

TABLE 9. – Mean Lifespans of Females From Biosatellite II and the Associated Postflight Experiments (Grosch, 1968 and 1970)

Biosatellite II Earth-based control		Flight		Postflight (phase C) Earth-based control		Vibrated and centrifuged	
Actual exposure, R	Lifespan, days	Actual exposure, R	Lifespan, days	Actual exposure, R	Lifespan, days	Actual exposure, R	Lifespan, days
			25.1±0.7				
1.7	23.3±0.9	0.7	[a]32.1±2.5	1.4	23.3±1.8	1.7	18.7±2.0
371	21.6±1.8	356	23.3±1.4	360	.....	352	.....
	17.2±2.7						
729	21.4±2.3	589	24.5±0.5	726	21.8±1.6	575	21.9±1.5
1245	23.8±1.9	1272	24.3±1.4	1241	21.6±1.9	1232	22.7±1.1
2431	24.9±1.5	2425	[a]34.1±4.4	2420	22.7±1.6	2471	21.3±1.8
[b]2000	[b]21.9±1.9	[b]2000	[b]24.2±1.5	.....	.....	.....	.....

[a] Received special care during the last week of life.
[b] Preirradiated with X-rays.

TABLE 10. – Frequency of Revertant Cells From a Requirement for Arginine

Nominal exposure, R	Estimated exposure, R	Number colonies	Number plates, *YEPD*[a]	Number revertants	Number plates, *−ARG*	Dilution difference	Revertants surviving cell
			Flight				
4000	1513	1518	10	3841	7	10^{-5}	0.361×10^{-4}
2000	918	1487	10	3553	10	10^{-5}	.238
1000	547	1604	10	2391	8	10^{-5}	.186
500	298	2092	10	2298	9	10^{-5}	.122
0	0.7	1685	10	1629	10	10^{-5}	.097
			Control I				
4000	1516	2120	10	7019	10	10^{-5}	0.331×10^{-4}
2000	899	1932	10	4154	8	10^{-5}	.269
1000	558	1981	10	3415	10	10^{-5}	.172
500	310	2004	10	2448	9	10^{-5}	.136
0	1.7	1985	10	938	5	10^{-5}	.095

[a] *YEPD* = Complete medium consisting of 1 percent yeast extract, 2 percent peptone, and 2 percent glucose.

remaining data increase with the exposure; thus, we attribute the "dip" in the control data to a chance fluctuation. Such a fluctuation was not seen in data obtained in the phase C experiment.

Artemia

The *Artemia* were placed in that part of each package that had originally been designed for shielded dosimeters. When it became apparent from preflight tests that shielded dosimeters were not needed, this part of the package was found to be suitable for monitoring certain other parameters. *Artemia* cysts were used in these positions since they had been found to have an enhanced adult survival after high-intensity, low-frequency vibrations.[3] The spaceflight did not significantly affect viability of the *Artemia* below the 5-percent level of significance, but there is a strong trend in the emergence data which might reflect the influence of spaceflight factors other than radiation (table 12).

The parameters that were measured, in addition to the emergence of the *Artemia* from the cysts, were the survival to adulthood and the fertility of every surviving individual; also, frequencies of embryologic abnormalities were recorded. In addition, an F_1 test was carried out which consisted of the measurement of frequencies of (1) zygotes encysted, (2) emergence, (3) fecundity, and (4) fertility. These data were obtained from pair matings of individuals that had survived to adulthood from the cysts that had been in the Biosatellite II experiment. By comparison of these data for the flight animals with those for animals that had been in the Earth-based control, we conclude, with the reservation cited above, that the encysted, quiescent form of the *Artemia* was not influenced significantly by the spaceflight or by the gamma radiation received during the flight. Furthermore, an appreciable preflight conditioning dose of gamma radiation (an exposure to 16 kR) did not aid in uncovering deleterious or beneficial influences of orbital spaceflight.

[3] Unpublished data from D. S. Grosch.

SUMMARY AND CONCLUSIONS

There are two major conditions of spaceflight that are difficult, if not impossible, to reproduce in the laboratory — weightlessness, as defined by a freely orbiting condition, and the vibration profile of a spacecraft during its launch and recovery phases. If without further experimentation and data analysis we arrange the differences found be-

TABLE 11. – Frequency of Red Variant Colonies Among Total Colonies Scored

Nominal exposure, R	Estimated exposure, R	Number colonies	Number red colonies	Number red sectors	Red colonies + red sectors	Red colonies + sectors / Total number colonies
			Flight			
4000	1513	19 817	48	32	80	4.0×10^{-3}
2000	918	20 559	43	36	79	3.8
1000	547	18 900	26	20	46	2.4
500	298	22 890	19	11	30	1.3
0	.7	20 087	17	7	24	1.1
			Control I			
4000	1516	25 488	51	43	94	3.7×10^{-3}
2000	899	16 732	16	12	28	1.7
1000	558	19 434	29	19	48	2.5
500	310	18 263	20	12	32	1.8
0	1.7	16 144	10	6	16	1.0

TABLE 12. – Emergence From *Artemia* Cysts in the Biosatellite II Experiment

[Unpublished data from D. S. Grosch]

Package	Radiation in flight, R	Percent emergence	*t* values	*P* values
Spaceflight	0.7	53.7	2.458	0.06
Earth-based control	1.7	45.3		
Spaceflight (pre-X-rayed 16 000 R)	2425	70.7	2.527	.05
Earth-based control (pre-X-rayed 16 000R)	2431	59.3		

tween animals in the spacecraft and animals in the Earth-based control (tables 13 and 14) in these two categories, we could come to the following conclusions.

Vibration

The enhancement of spontaneous recessive lethal frequencies in sperm of *Habrobracon* seen from analysis of the flight animals was reproduced during the phase C test, in which the flight vehicle was vibrated and centrifuged; this effect thus seems clearly to have been induced by something other than weightlessness. The enhancement of embryonic, larval, and pupal deaths in the nonirradiated flight females (deduced by genetic reconstruction to have been caused by chromosomal aberrations and recessive lethal mutations) was restricted to their placement at different positions within a package. Althought the effect was not seen in the postflight vibration and centrifugation test, we believe that the "position effect" nature of the phenomenon excludes an effect from weightlessness. The strong trend for enhanced emergence from *Artemia* cysts might be caused by vibration.

Weightlessness

At this time, the best candidates for an effect of weightlessness appear to be the following: (1)

TABLE 13. – Genetic Effects Obtained in the *Habrobracon* Experiment in Biosatellite II Compared With Those Obtained on Earth

End points	Effect of flight dynamics	
	Flight alone	Flight plus radiation
Sperm:		
Dominant lethal mutations	Null	Null
Recessive lethal mutations	Increased	Null
Translocations	Null	Null
Oocytes:		
Hatchability:		
Metaphase I	Reduced	Reduced
Late prophase I	Null	Null
Early prophase I	Null-reduced	Reduced
Recombination: Late prophase I	Null	Null
Transitional oogonia: Hatchability	Null	Increased
Primitive oogonia: Hatchability	Null	Increased

TABLE 14. – Physiologic Effects Obtained in the *Habrobracon* Experiment in Biosatellite II Compared with Those Obtained on Earth

End points	Effect of flight dynamics	
	Flight alone	Flight plus radiation
Survival:		
Males	Null	Null
Females	Null	Null
Fecundity:		
Transitional oogonia	Null	Increased
Primitive oogonia	Null	Increased
Behavior:		
Mating of males	Disoriented	Disoriented
Oviposition of females	Null	Null
Other effects:		
Fertilizing capacity of sperm	Null	Enhanced
Xanthine dehydrogenase activity in males	Reduced	Reduced
Lifespan of females	Increased	Increased

enhancement of fecundity and hatchability of primitive and transitional oogonia; (2) disorientation of male mating behavior; (3) increased lifespan of females; and (4) decreased xanthine dehydrogenase activity. The two remaining effects of flight, namely, the enhanced fertilizing capacity of sperm and the reduced hatchability of eggs from females irradiated as metaphase I and early prophase I oocytes, could be either from weightlessness or from other factors of spaceflight.

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